

TCACAAGAATCGAACCATGTAGAGAG -855

AP-1 site **CCAAT box**
ACTTAGTTGTCTTTTAACAGA**ATTGGG**CACGGGCTGTTTCAGAAACAACAATCTTTCACAT -795

CCATTATAATGATAGCATTAGTGTAGTTTGTTTAGCAAATGTTTACTG**TGAGCCTG**TAT -735

GTGCTGAGCCTGCTATGTAAGAAGTGTGGCTCTCTGGACAGGAGACAGAATACTAAACAA -675

CACAAC**TACTGATCTTTGGCTGCCTGGCATGCTTCTCACTTCATATGGTATCAGCAATT** -615

TAGCACCACAAACGTCCTTTAGAGAACCAGCCCTTTCTCATTCTTGGTTCTAGTGGCTTG -555

AGTAGACTGACCCAGCCTACCCAAAGTGGATTGACTCCTAGCAATTCATTAATCTAGC -495

CCAAT box
CCAATAAATGTCAAGTACAGGACTTTTATTGAAAGCATTTCAGAAAAGAGGTGGACTCTC -435

ACACTAAACATTTGTA**ACTAAATAAGGGATGTTAGAAATT**CTCTAGAAAGGAAGCTATGA -375

TATAAATGGGTTGCTAGATGGGTCTAGTAGATGGTGGCC**GTGCTT**GTGTACTGCCTTGT -315

GTATTGTGCTACCATAGCCCTCCCAAACTGTACTCTGGCTCCTGGCATTTCGGTCTCTT -255

CAACCAGATGGTCAGCTCTCTAAGTGAAGGAGACACATCTCCAACATGCTTGGTTCTAGC -195

ACAACAGAAGGGCTCAAACACATACCTGCT**AAAGAACTATCCTGATGGATTAGCAGCA** -135

Inverted CCAAT Box/E2F-like site
TGGCCATGAGGC**ATTGGCGGT**CTATCACTGGGA**ACTCAGGTTTCTGGTGCTCCAGTACC** -75

TCTACTGGCTGATACCACATCCTACA**ATTCACTTCATAGGCTTGGGTTCTGCTCTGGGC** -15

GC-rich motif

TGAATAGGTGGTCCACTCTGAGTCATCAGCTGTGGGTGATGATGTGGTCACTGCATGATT 46

CTCACACAAGCACCCAGAGG**ACGTCATCAGGCAGAGGCAGTGGGGGTGGGCAGCATTAC** 106

Start of GrB-NIC cDNA

AGAAAATC**TGTGATGAGACACCACAAACCAGAGGGGAACATGAAGTC**ACTGAGCCTGCT 166

CBF/AP-1 **CRE** **Ikaros**

AGAAAATC**TGTGATGAGACACCACAAACCAGAGGGGAACATGAAGTC**ACTGAGCCTGCT 7

(GrB)TATA box **NF-AT site**

CCACCTCTTTCCTCTCCCAAGAGCTAAAGAGAGCAAGGAGGAAACAACAGCAGCTCCAA 226

H L F P L P R A K R E Q G G N N S S S N 27

Start of human CTL GrB cDNA

CCAGGGCAGCCTTCCTGAGAAGATGCAACCAATCCTGCTTCTGCTGGCCTTCCTCCTGCT 286

Q G S L P E K **M** Q P I L L L L A F L L L 47

CCCCAGGGCAGATGCAGGGGAGATCATCGGGGACATGAGGCCAAGCCCCACTCCCCGCC 346

P R A D A **G E** I I G G H E A K P H S R P 67

CTACATGGCTTATCTTATGATCTGGGATCAGAAGTCTCTGAAGAGGTGCGGTGGCTTCT 406

Y M A Y L M I W D Q K S L K R C G G F L 87

GATACAAGACGACTTCGTGCTGACAGCTGCTCACTGTTGGGGAAGCTCCATAAATGTCAC 466

I Q D D F V L T A A H C W G S S I N V T 107

CTTGGGGGGCCCAATATCAAAGAACAGGAGCCGACCCAGCAGTTTATCCCTGTGAAAAG 526

L G A H N I K E Q E P T Q Q F I P V K R 127

ACCCATCCCCATCCAGCCTATAATCCTAAGAAGTCTCCAACGACATCATGCTACTGCA 586

P I P H P A Y N P K N F S N D I M L L Q 147

GCTGGAGAGAAAGGCCAAGCGGACAGAGCTGTGCAGCCCTCAGGCTACCTAGCAACAA 646

L E R K A K R T R A V Q P L R L P S N K 167

GGCCAGGTGAAGCCAGGGCAGACATGCAGTGTGGCCGGCTGGGGGCAGACGGCCCCCT 706

A Q V K P G Q T C S V A G W G Q T A P L 187

GGGAAACACTCACACACTACAAGAGGTGAAGATGACAGTGCAGGAAGATCGAAAGTG 766

G K H S H T L Q E V K M T V Q E D R K C 207

CGAATCTGACTTACGCCATTATTACGACAGTACCATTGAGTTGTGCGTGGGGGACCCAGA 826

E S D L R H Y Y D S T I E L C V G D P E 227

GATTAAAAAGACTTCCTTTAAGGGGACTCTGGAGGCCCTCTTGTGTGAACAAGGTGGC 886

I K K T S F K G D S G G P L V C N K V A 247

CCAGGGCATTGTCTCCTATGGACGAAACAATGGCATGCCTCCACGAGCCTGCACCAAAGT 946

Q G I V S Y G R N N G M P P R A C T K V 267

CTCAAGCTTTGTACACTGGATAAAGAAAACCATGAAACGCTACTAACTACAGGAAGCAAA 1006

S S F V H W I K K T M K R Y * 281

CTAAGCCCCGCTGTAATGAACACCTTCTCTGGAGCCAAGTCCAGATTTACACTGGGAG 1066

AGGTGCCAGCACTGA**TATAA**TACCT 1092

Figure 1

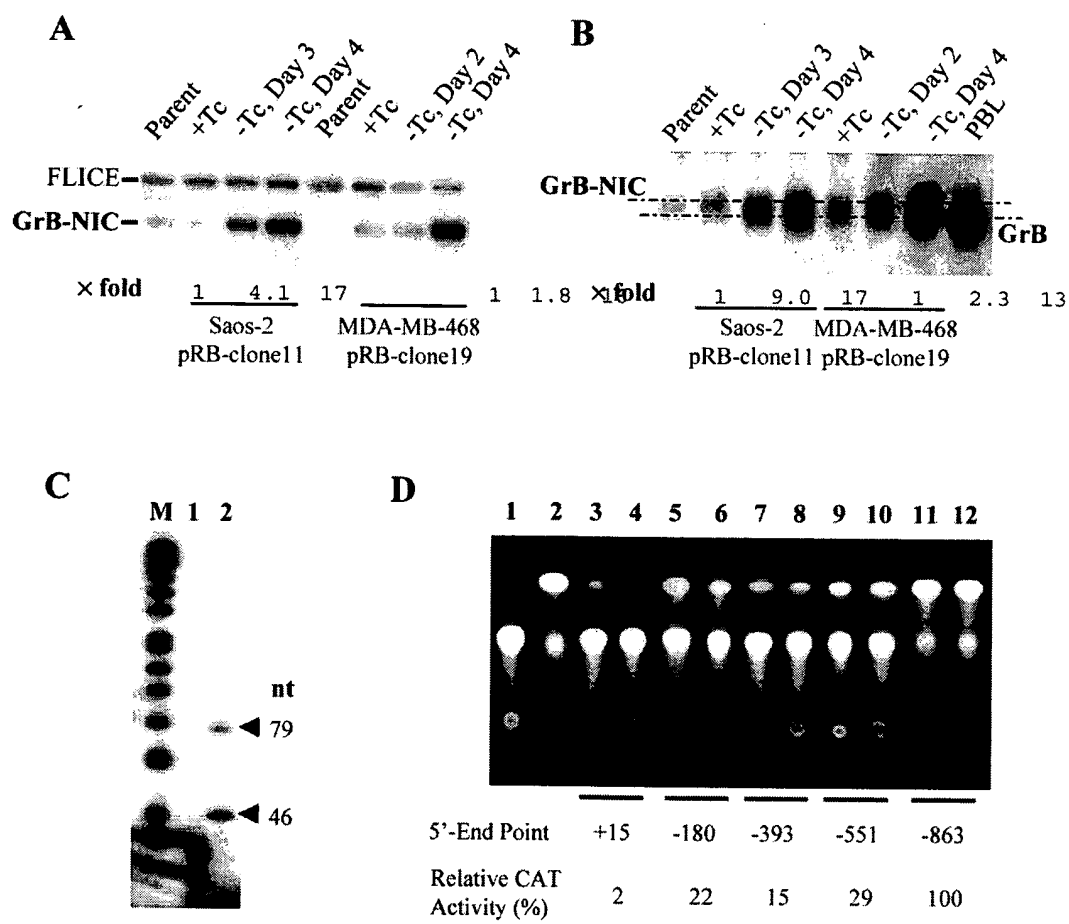


Figure 2

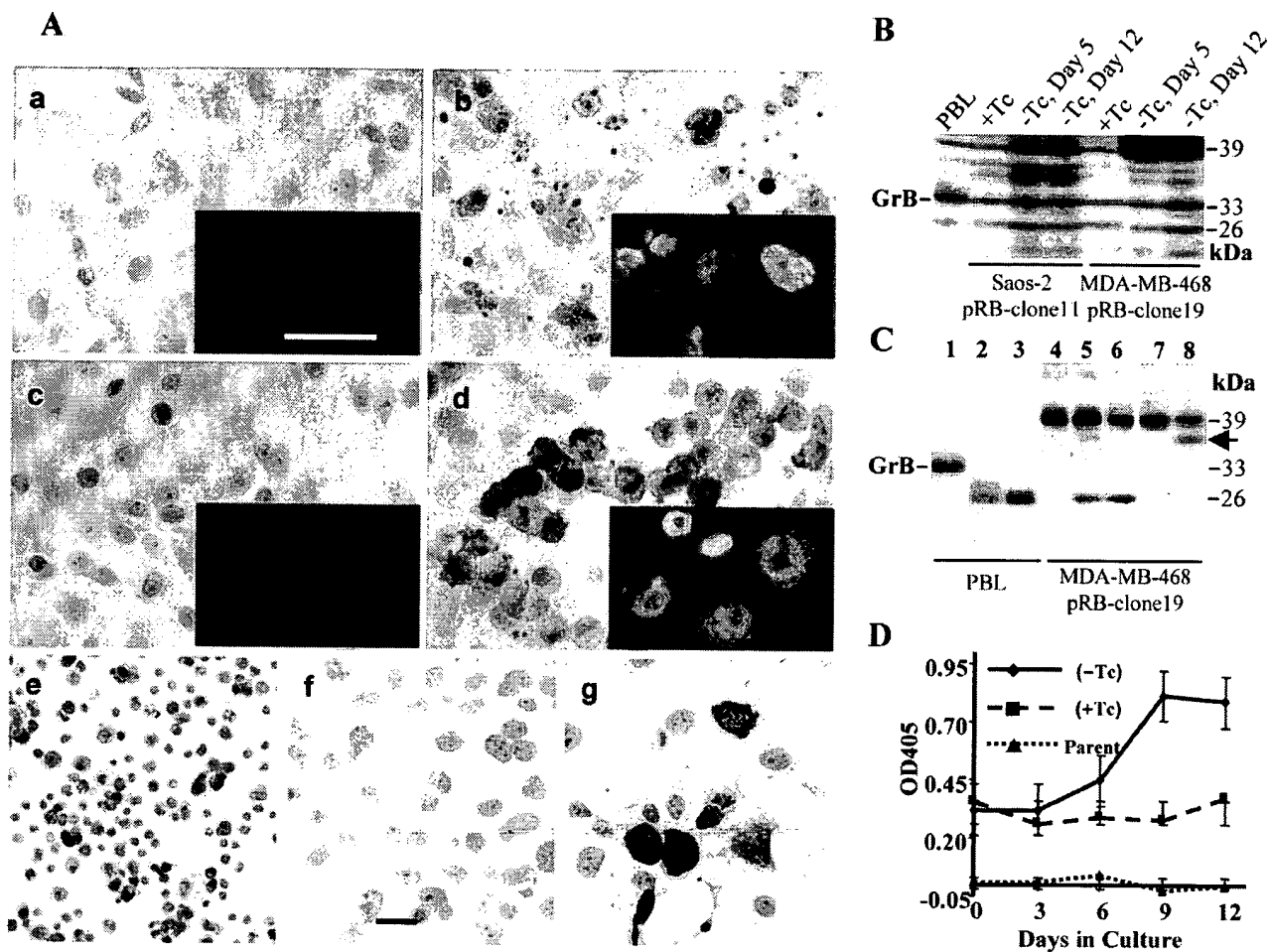


Figure 3

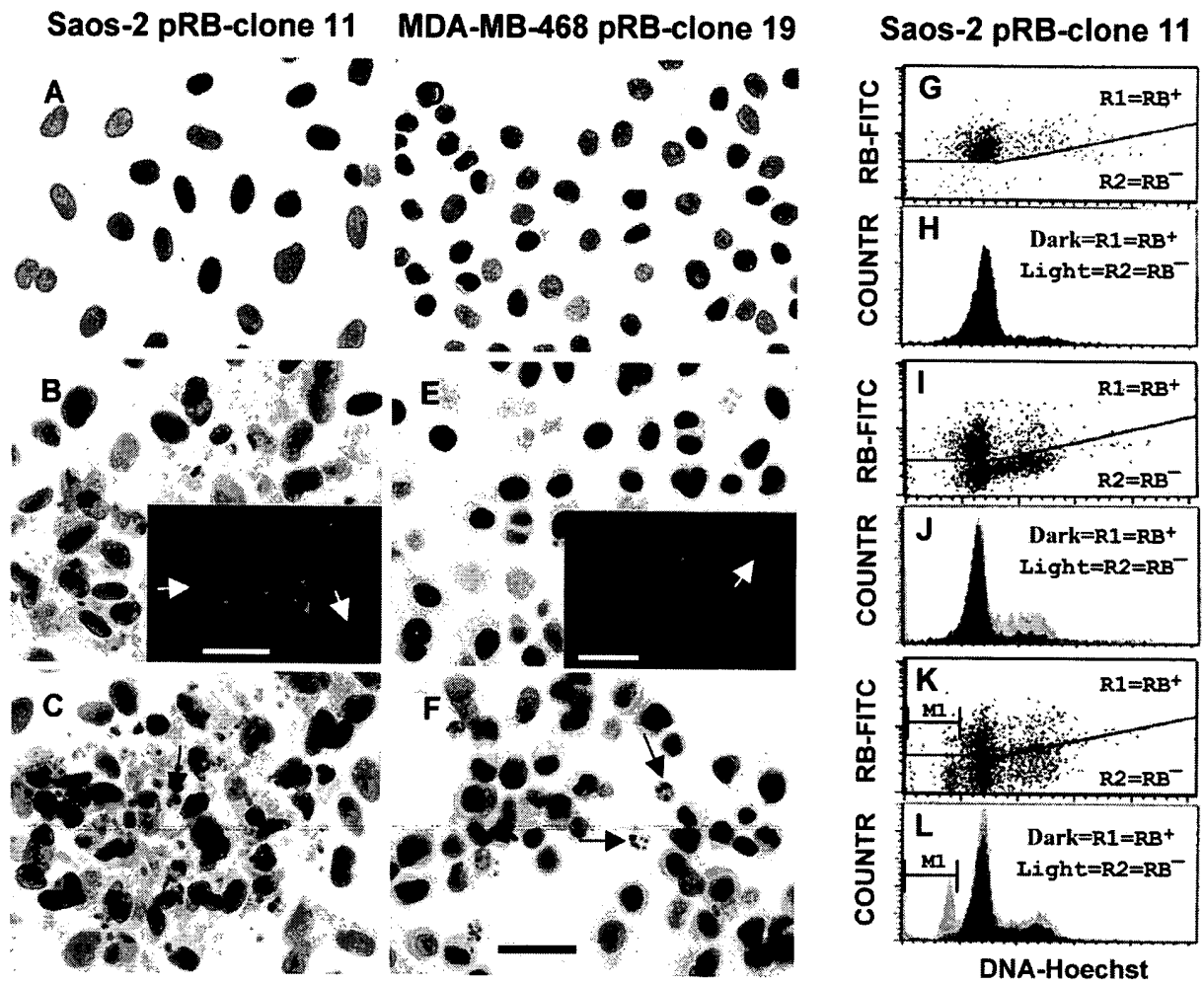


Figure 4

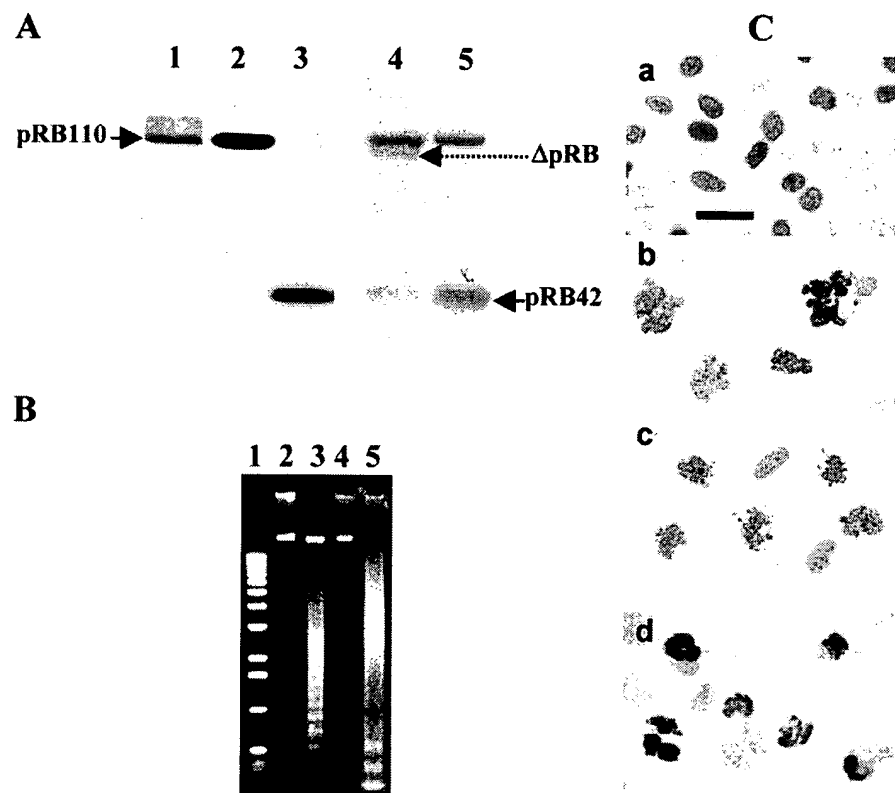


Figure 5

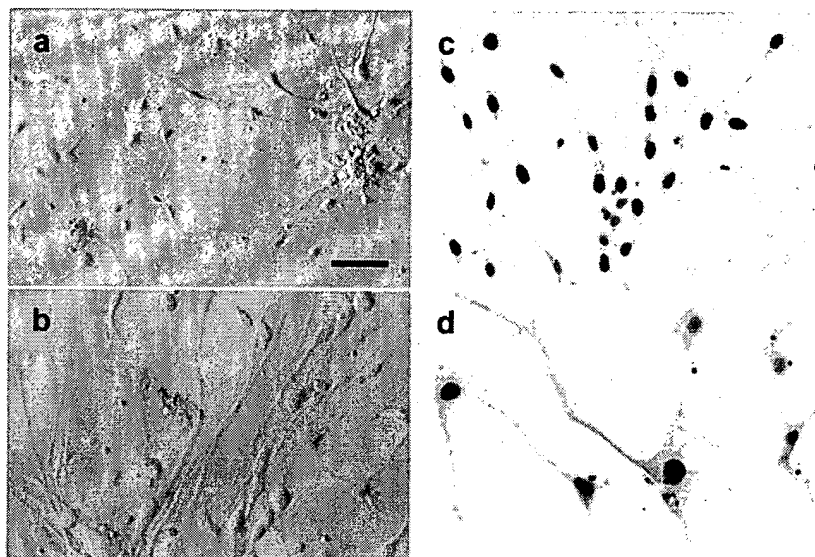


Figure 6

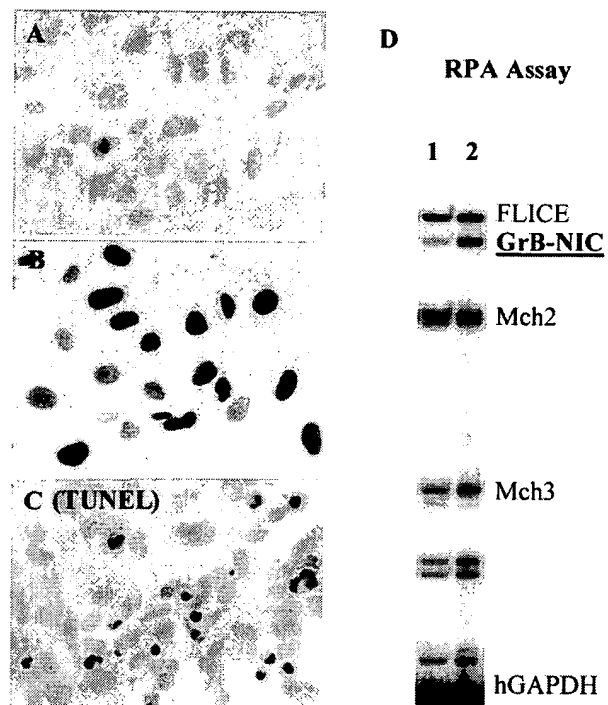


Figure 7

MDA-MB-468 pRB-clone 19

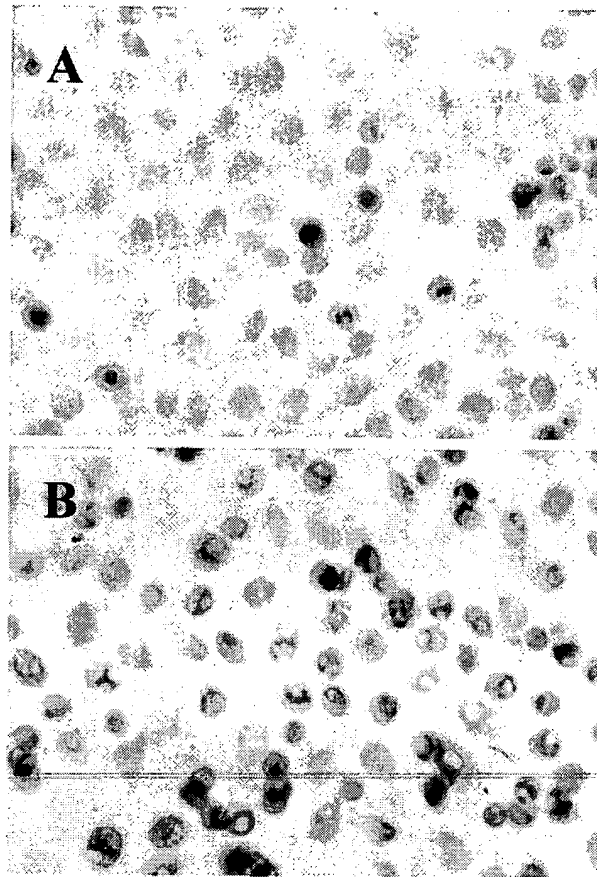


Figure 8

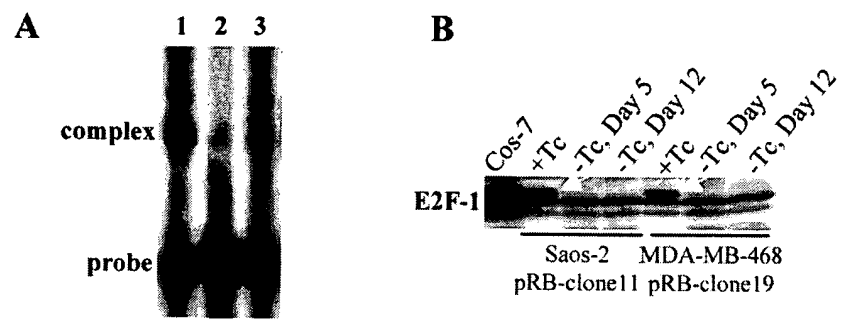


Figure 9

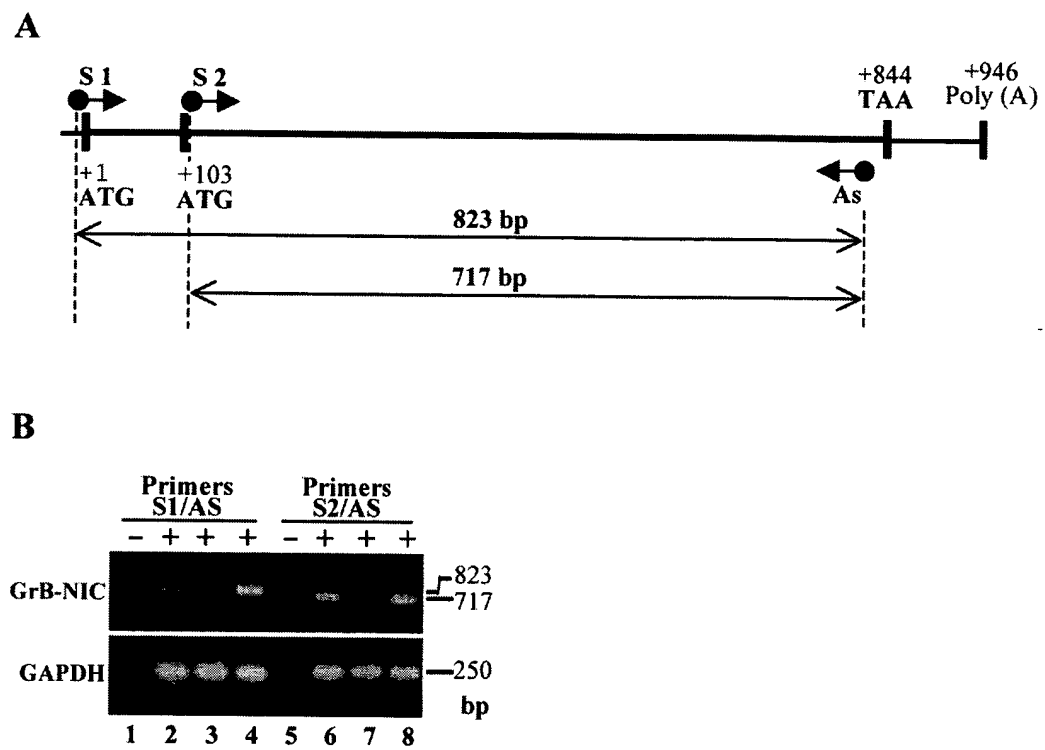


Figure 10

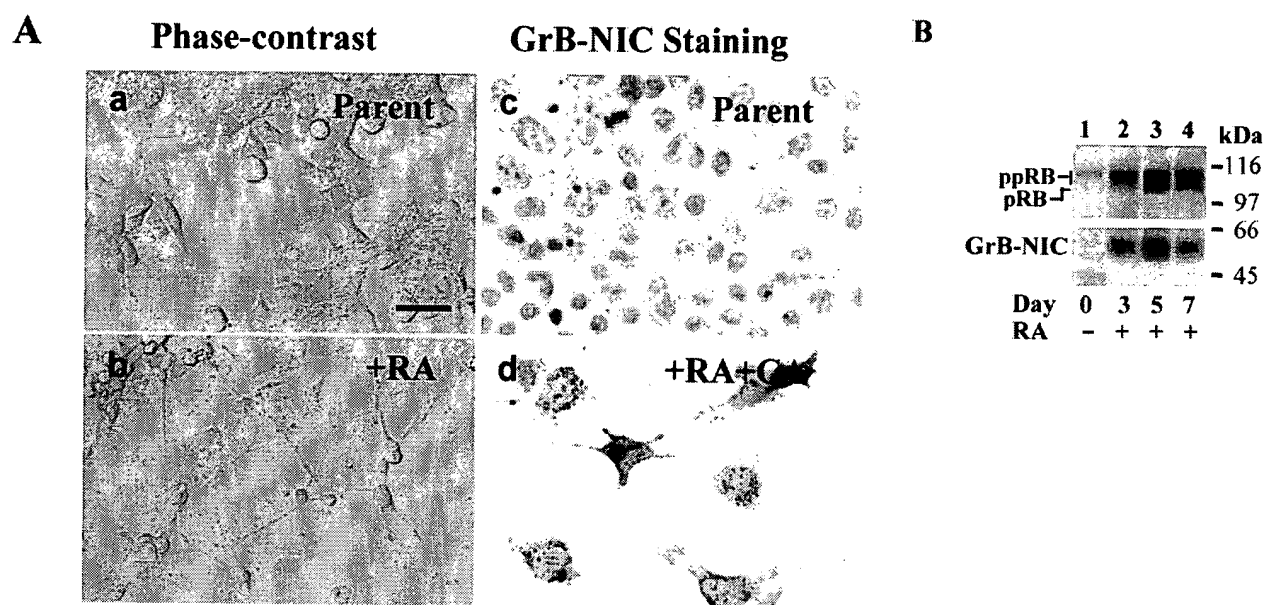


Figure 11

Breeding Scheme:

The RB and GrB-NIC double-mutant mouse embryos extended survival to approximately embryonic day 19.5

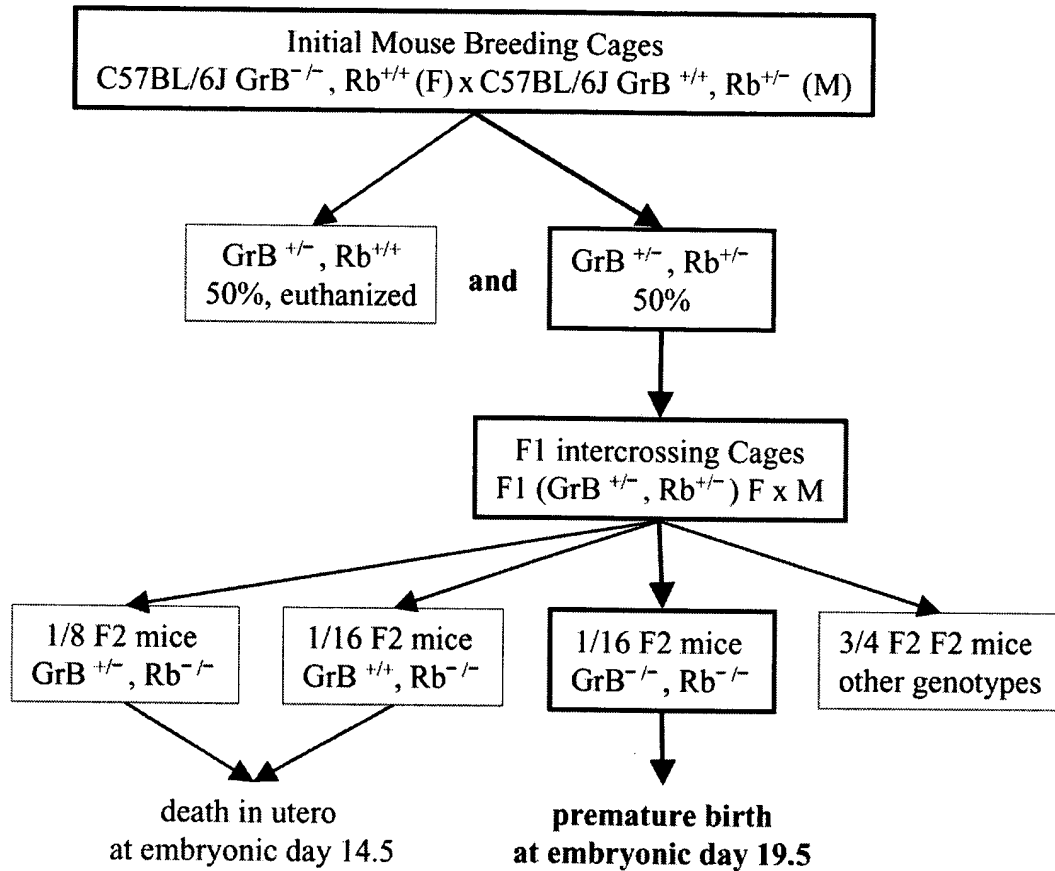


Figure 12

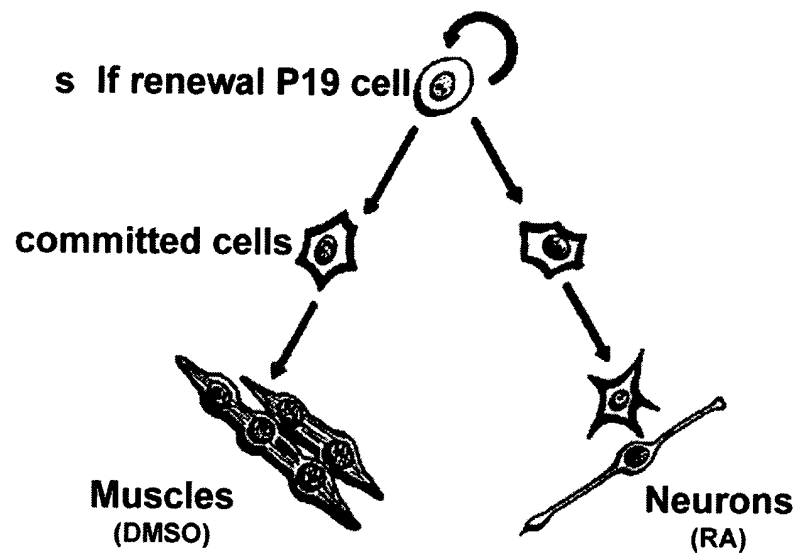
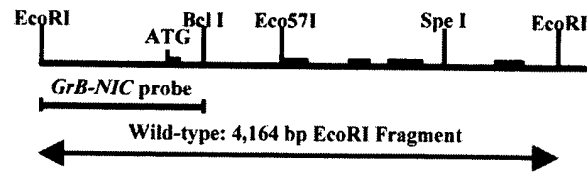


Figure 13

Genomic Map of the Murine GrB-NIC (GrB) Gene



Structure of the Targeting Vector



Schematics of the Disrupted GrB-NIC (GrB) Allele

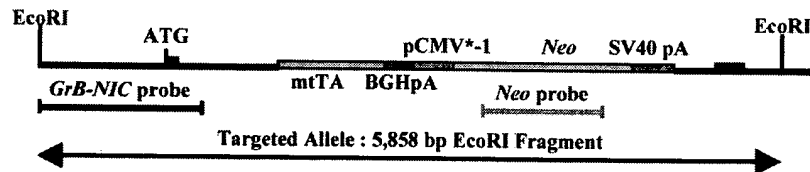


Figure 14

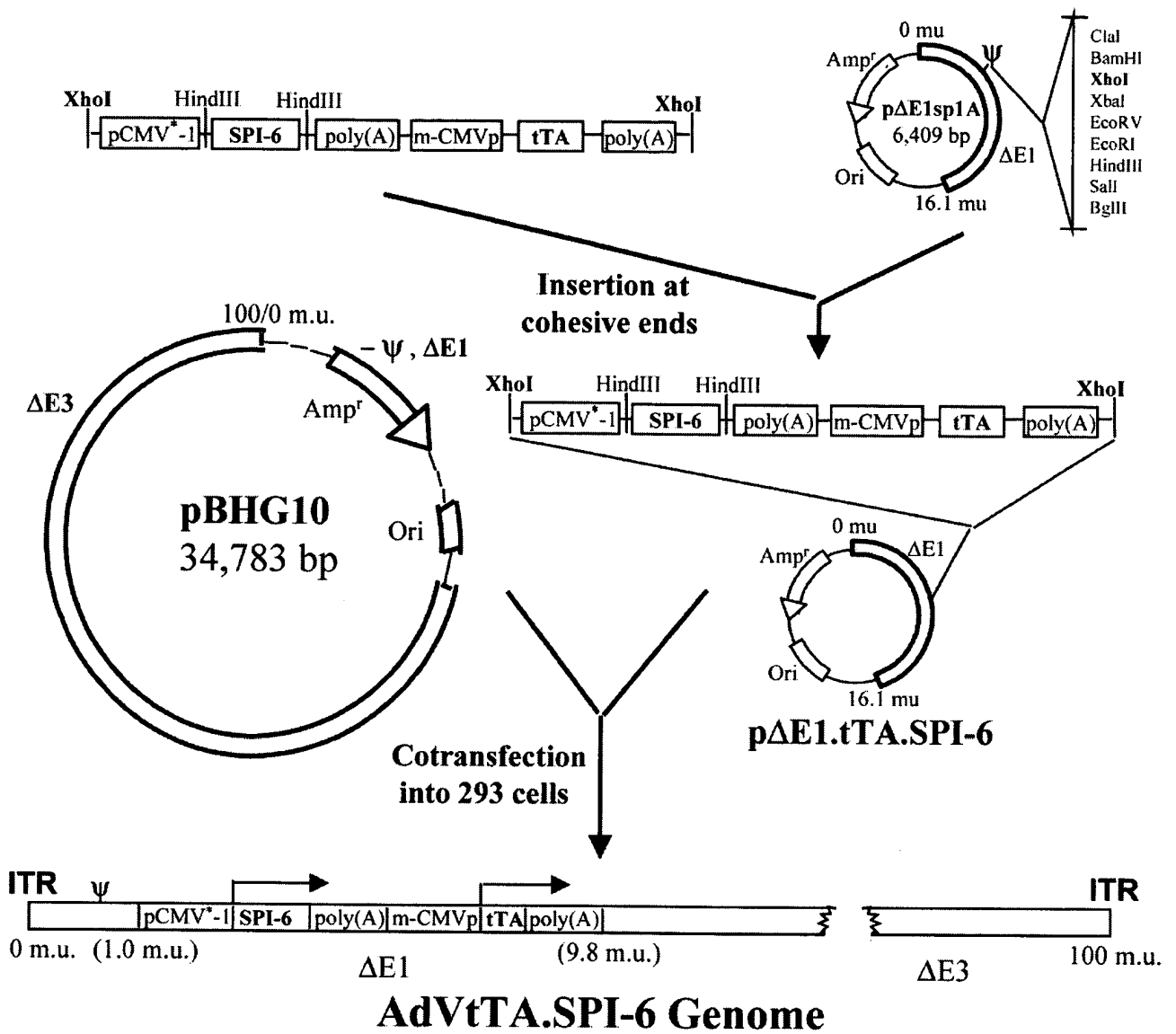


Figure 15

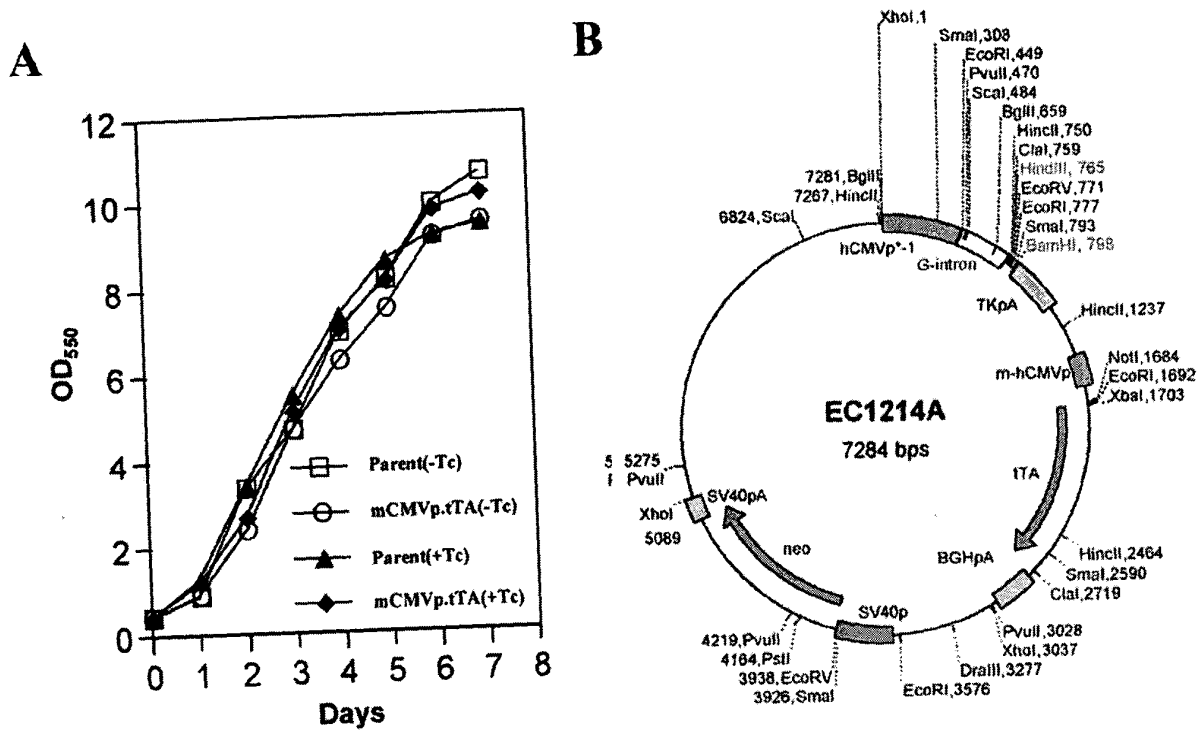


Figure 16

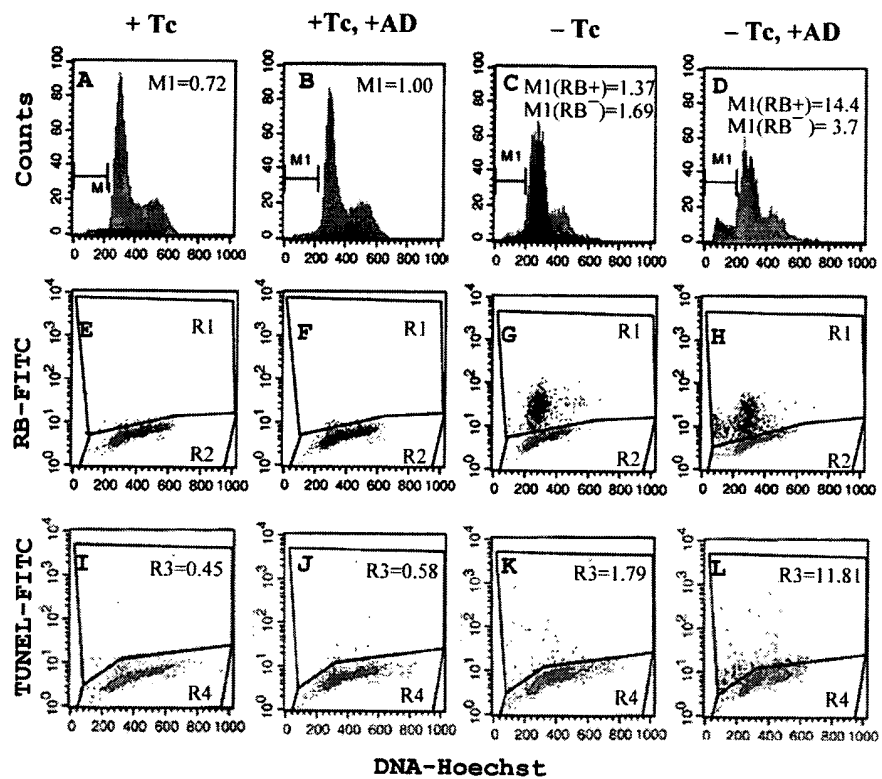


Figure 17

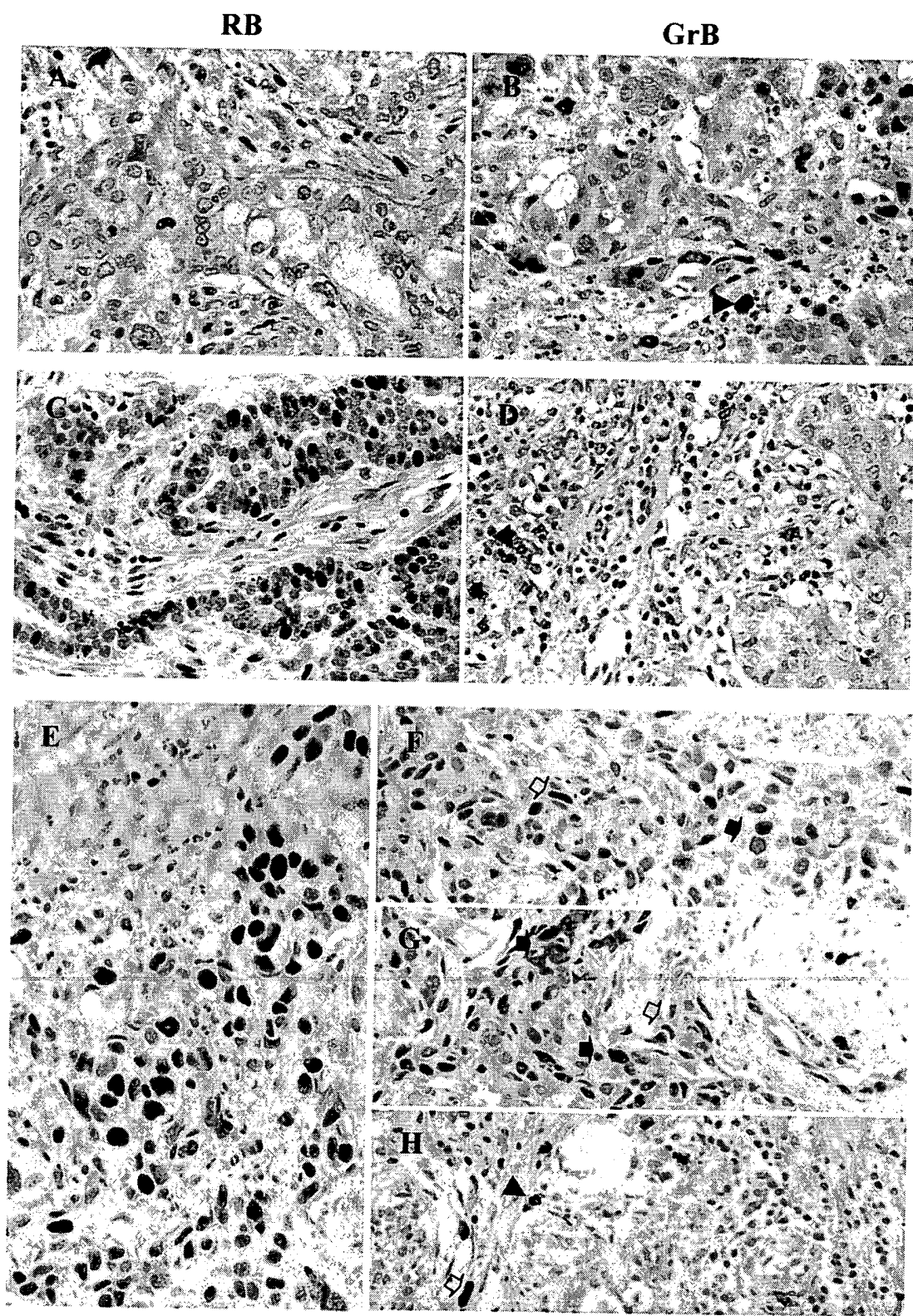


Figure 18

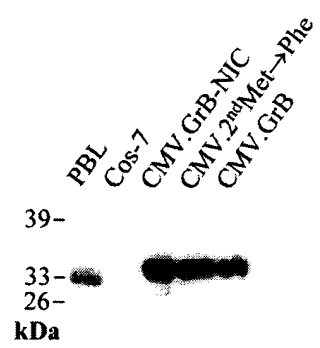
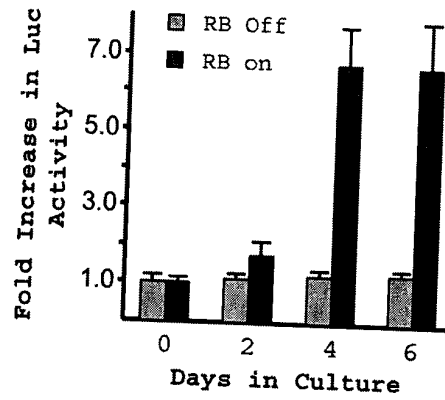


Figure 19

A

Saos-2.pRB clone with chromosomally
integrated pGrB-NIC-Luc

**B**

MC3T3 Stably Transfected
with pGrB-NIC-Luc

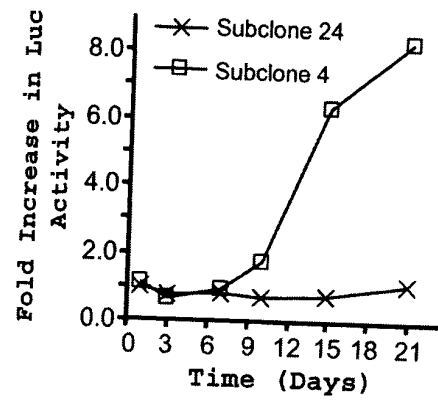


Figure 20